

Clec5a Cas9-CKO Strategy

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Reviewer :

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2019-11-14

Project Overview



Project Name

Clec5a

Project type

Cas9-CKO

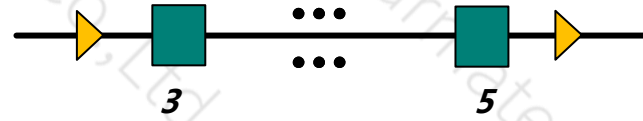
Animal background

C57BL/6JGpt

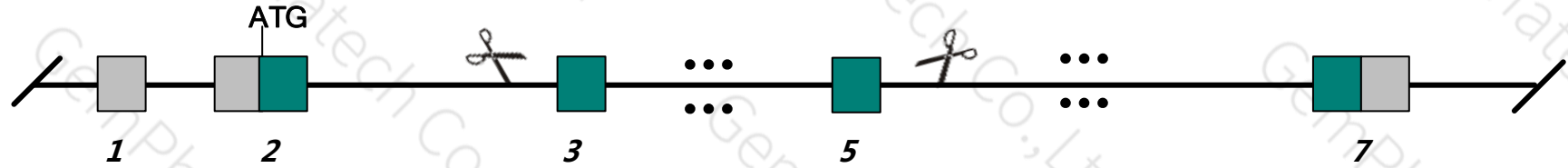
Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Clec5a* gene. The schematic diagram is as follows:

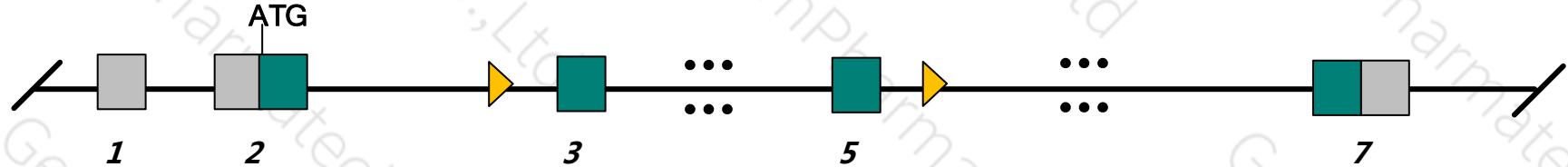
Donor and CRISPR/Cas9 System



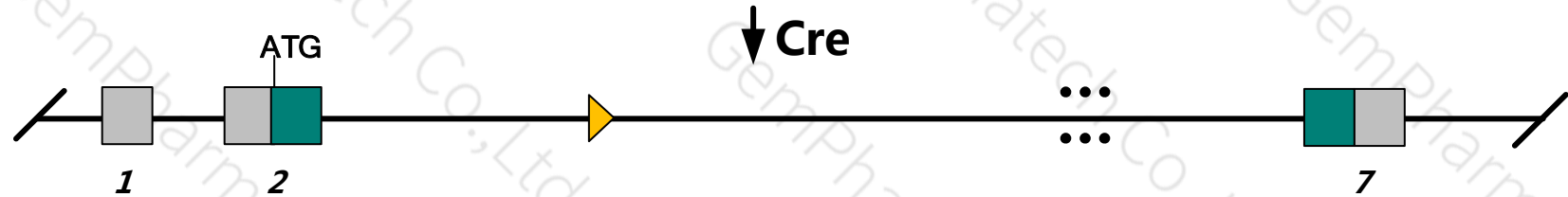
Wild-type allele



Conditional KO allele



KO allele



Cas9/sgRNA



Uncoding region



Coding region



LoxP

Technical routes

- The *Clec5a* gene has 4 transcripts, According to the structure of *Clec5a* gene, exon3-exon5 of *Clec5a-203* transcript is recommended as the knockout region. The region contains the 272bp coding sequence. Knock out the region, result in destruction of protein.
- This project uses CRISPR/Cas9 technology to modify *Clec5a* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed, Cas9, sgRNA and donor were microinjected into fertilized eggs of C57BL/6JGpt mice and homologous recombination was carried out to obtain F0 mice. A stable and hereditary F1 generation mouse model was obtained by mating F0 generation mice with C57BL/6JGpt mice which were confirmed positive by PCR-sequencing.
- The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

- The *Clec5a* gene is located in the Chr6. If the knockout mice are mixed with other mice, two target genes are avoided on the same chromosome as possible, otherwise the offspring of mice with double gene positive and homozygous gene knockout can not be obtained.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)



Clec5a C-type lectin domain family 5, member a [*Mus musculus* (house mouse)]

Gene ID: 23845, updated on 18-Apr-2019

Summary

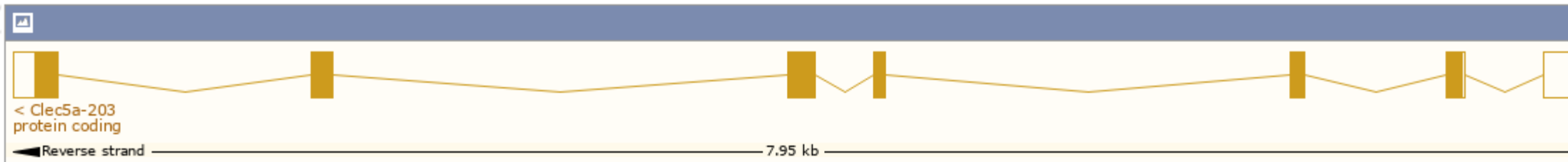
Official Symbol	Clec5a provided by MGI
Official Full Name	C-type lectin domain family 5, member a provided by MGI
Primary source	MGI:MGI:1345151
See related	Ensembl:ENSMUSG00000029915
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Mdl1; Ly100; MDL-1; Clec5f5
Expression	Biased expression in liver E18 (RPKM 1.9), liver E14 (RPKM 1.1) and 8 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

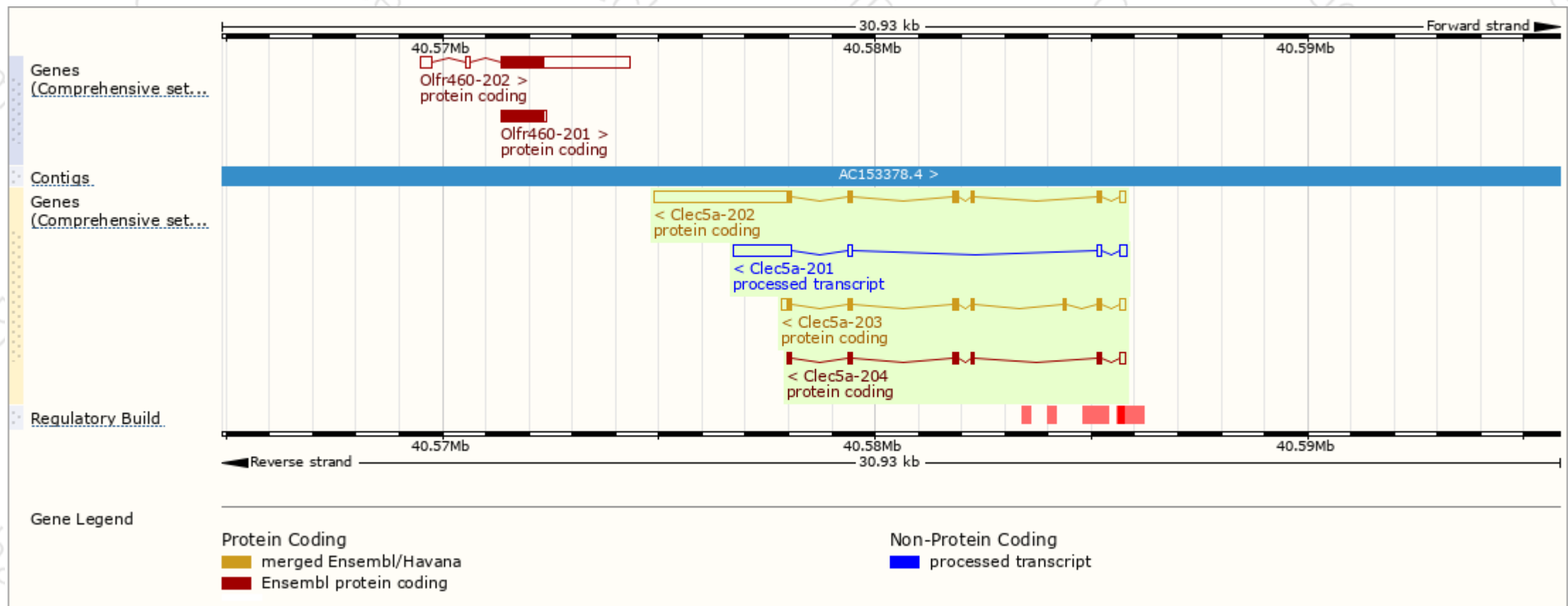
The gene has 4 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Clec5a-202	ENSMUST00000101491.10	3747	165aa	Protein coding	CCDS20037	Q9R007	TSL:1 GENCODE basic APPRIS ALT2
Clec5a-203	ENSMUST00000129948.8	858	190aa	Protein coding	CCDS20036	Q9R007	TSL:1 GENCODE basic APPRIS P4
Clec5a-204	ENSMUST00000177178.1	665	164aa	Protein coding	-	A0A0R4J269	TSL:1 GENCODE basic APPRIS ALT2
Clec5a-201	ENSMUST00000031975.6	1745	No protein	Processed transcript	-	-	TSL:1

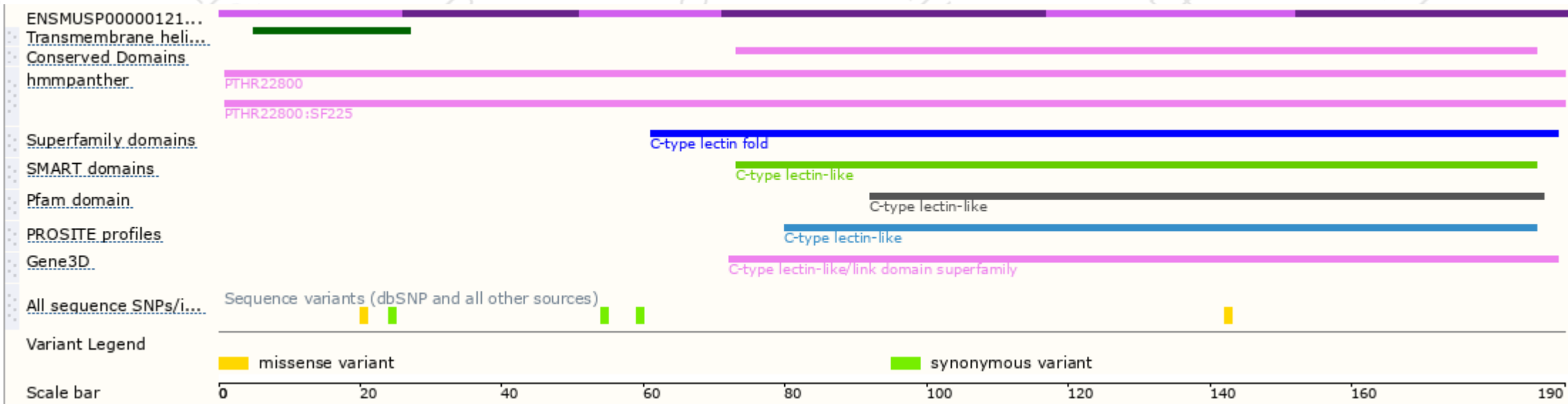
The strategy is based on the design of *Clec5a-203* transcript, The transcription is shown below :



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.

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